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# In Vitro and In Vivo Evaluation of Ceftezole, a New Cephalosporin Derivative

MINORU NISHIDA,* TAKEO MURAKAWA, TOSHIAKI KAMIMURA, NAOHIKO OKADA, HIROSHI SAKAMOTO, SHIGEMI FUKADA, SHOJI NAKAMOTO, YOSHIKO YOKOTA, AND KAZUYO MIKI

*Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan*

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Ceftezole, a new cephalosporin derivative, was compared with cefazolin, cephaloridine, and cephalothin. Data obtained indicate that it is a broad-spectrum antibiotic, with almost identical antimicrobial activity against pathogenic organisms isolated from patients. The therapeutic effect of ceftezole on experimental infections in mice was similar to that of cefazolin and was superior to that of cephalothin. The binding of ceftezole to serum proteins was somewhat less than that of cefazolin. The concentrations of ceftezole in the sera of test animals and human volunteers were determined after intramuscular injection of 20 mg/kg and after a single dose of 500 mg, respectively. The concentration of ceftezole in the serum of volunteers peaked at 24.9 $\mu$g/ml 15 min after injection and remained effective (about 2.6 $\mu$g/ml) at 4 h. The half-life in serum under the same conditions was 56 min, i.e., about one-half that of cefazolin. The 24-h urinary recovery rate was 87.5%. Most of the administered ceftezole was excreted unchanged mainly through the urinary tract. The biliary excretion rate in SD strain rats after intramuscular injection of 20 mg/kg was about 4.4%. As compared with commercially available cephalosporins, ceftezole was second only to cefazolin in biliary excretion rate. Various tissue levels of ceftezole in animals were higher than cephalothin but, with the exception of renal levels in the early stage after administration, were lower than cefazolin.

During the screening of new cephalosporin derivatives in Fujisawa Research Laboratories, cefazolin was identified as an agent of outstanding potential. It has since been proved to have excellent antimicrobial activity and tolerance (4) and has been widely tested in clinical situations (1-3).

In subsequent screening, some potentially more useful derivatives were found among those closely related to cefazolin in chemical structure. Of these, ceftezole (Fig. 1) has recently been developed and is reported here.

In the present study, the in vitro and in vivo antimicrobial activity, absorption, excretion, and tissue distribution of ceftezole were investigated and compared with those of related cephalosporins.

## MATERIALS AND METHODS

**Test antibiotics.** Ceftezole and cephalosporin C were provided by Fujisawa Research Laboratories, cefazolin was provided by Fujisawa Pharmaceutical Co., Ltd., and cephaloridine and cephalothin were provided by Eli Lilly & Co.

**Subjects.** The following subjects were included: male ICR strain mice, each weighing 17 to 23 g, 4 weeks of age; male SD strain rats, each weighing 170 to 245 g, 6 weeks of age; male beagle dogs, each weighing 9.5 to 12.5 kg; male rhesus monkeys, each weighing 6.4 to 9.9 kg; and healthy male adult volunteers, each weighing 55 to 75 kg.

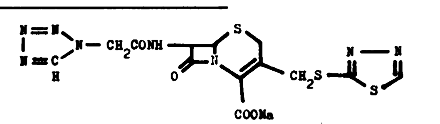

FIG. 1. *Chemical structure of ceftezole. Sodium (6R, 7R)-3-[(1,3,4-thiadiazol-2-yl)-thiomethyl]-8-oxo-7-[2-(1H-tetrazol-1-yl) acetamido]-5-thia-1-azabicyclo-[4,2,0] oct-2-ene-2-carboxylate. ($C_{13}H_{11}N_8O_4S_3Na$: molecular weight, 462.47).*

**Measurement of in vitro antimicrobial activity.** The in vitro antimicrobial activity of the test antibiotics was determined by the agar dilution method. An overnight broth culture and decimal dilutions thereof were spot-inoculated, using a multiple inoculator onto heart infusion agar (Difco) containing graded concentrations of the test drugs. The minimum inhibitory concentration (MIC) was estimated after incubation at 37 C for 20 h. For testing of activity against three species of *Streptococcus* and against *Corynebacterium diphtheriae*, 10% rabbit blood was added to the above media.

**Bactericidal activity.** Heart infusion broth containing one-fourth the MIC, the MIC, and four times the MIC of each test drug was inoculated with *Escherichia coli* NIHJ JC-2 in a quantity sufficient to yield a final concentration of $10^6$ cells/ml. The cultures were then incubated at 37 C with shaking for an initial 8 h and without shaking for a subsequent 16-h period. The number of viable cells was measured at regular intervals throughout the incubation period.

**Protein binding. (i) Ultrafiltration method.** To 4.5 ml of human serum (Consera) or of fresh serum from each animal, 0.5 ml of antibiotic solution (300 $\mu$g/ml) in M/15 phosphate buffer (pH 7.0) was added and incubated at 37 C for 1 h. The mixture was poured into a Visking tube (8/32 in size) hung in a 15-ml polypropylene tube and centrifuged at 1,000 × *g* for 30 min. The concentration-free antibiotic in the ultrafiltrate was determined by the disk method. When these values are expressed as $X$ and the values obtained in the reference experiment with buffer in place of serum are expressed as $Y$, the binding rate $(B)$ of the antibiotics was determined as follows:

$$B\ (\%) = \frac{Y - X}{Y} \times 100$$

**(ii) Effect of buffer dilution on antibiotic-protein binding.** The antibiotic solution (500 $\mu$g/ml) was mixed with ninefold volumes of human serum (Consera). The mixture was allowed to stand at room temperature for 1 h, and then was diluted two-, four-, and eightfold with M/15 phosphate buffer (pH 7.0). The concentration of antibiotic in each dilution was determined by the disk method. When M/15 phosphate buffer was used instead of serum, the concentration of antibiotic was expressed in terms of relative value as 100.

**Protective effect on infections in mice.** Male ICR white mice, 4 weeks of age and each weighing 17 to 23 g, were used in groups of 10 mice each. The organisms were cultivated overnight at 37 C on brain heart infusion agar and were then suspended in 2 or 5% mucin solution to obtain microbial cell concentrations of $1 \times 10^6$ to $4 \times 10^6$/ml. Mice were inoculated intraperitoneally with 0.5 ml of the suspension. Each of the test antibiotics was administered subcutaneously in varying dosages to a group of 10 mice, 1 h after challenge. The mean effective dose ($ED_{50}$) values were found by the probit method from the number of surviving animals after 2 weeks of observation.

**Microbiological assay.** Each 10 ml of agar medium (1% sodium citrate, 0.5% polypeptone, 0.3% meat extract, and 1% agar) was inoculated with $10^5$ spores of *Bacillus subtilis* ATCC 6633 per ml and placed in petri dishes. Test disks (6 or 8 mm in diameter were immersed in the standard solutions or test solutions containing the antibiotics under study. After removing excess water, the disks were placed on the above media. After standing at 37 C for 20 h, the diameters of the inhibitory zones were measured.

**Absorption and excretion. (i) Drug concentrations in serum.** The test drugs were given intramuscularly in a dose of 20 mg/kg to experimental animals and in a dose of 500 mg to healthy human volunteers. Groups of 10 rats each were anesthetized with chloroform, and blood was collected from the heart at 0.25, 0.5, 1, 1.5, and 2 h. The blood was collected from dogs, monkeys, and human volunteers from the foreleg veins at 0.5, 1, 2, 4, and 6 h. The concentrations of antibiotic in each serum sample was determined by the disk method, using standard solutions prepared with sera of respective animals and volunteers.

**(ii) Urinary excretion.** Urine of rats was collected with a urine collector at 0 to 3, 3 to 6, and 6 to 24 h after intramuscular injection of 20 mg of the test drugs per kg. Urine was collected from dogs and monkeys with a catheter at specified intervals after intramuscular injection. The concentration of antibiotic in each urine sample was bioassayed with the standard buffer at pH 7.0, and the urinary recovery rate was calculated.

**(iii) Identification of active substances excreted into urine.** Ceftezole was given intramuscularly to experimental animals (20 mg/kg) and human volunteers (500 mg). The urine was collected over a period of 6 h after administration and examined by thin-layer chromatography and bioautography. The standard ceftezole solution (1 mg/ml) and urine samples were examined chromatographically by the use of Eastman Chromagram sheet no. 6061. The sheet was then dried and developed with the solvent system *n*-butanol-acetic acid-water (4:1:5, top layer). The dried sheet was then placed on an agar plate that had been seeded with 0.2% of the spore suspension ($2 \times 10^8$ spores/ml) of *B. subtilis* ATCC-6633.

**(iv) Biliary excretion.** Rats anesthetized with pentobarbital were fixed in a supine position, and a polyethylene cannula was inserted into the bile duct. Bile samples were collected at 0 to 3, 3 to 6, and 6 to 24 h after intramuscular injection of 20 mg of the test drugs per kg. The antibiotic levels in the bile samples were assayed with the standard solutions prepared with M/15 phosphate buffer (pH 7.0).

**(v) Tissue distribution.** Groups of three rats each received intramuscularly 20 mg of each of the test antibiotics per kg and were sacrificed at 30, 60, and 90 min after drug administration. Liver, kidneys, lungs, heart, and spleen were removed and after light washing in saline solution, the organs from each group of animals were pooled, mixed with ethanol, and homogenized in a polytron homogenizer. The concentrations of antibiotic in the supernatants obtained by centrifuging the tissue homogenates at 10,000 rpm for 10 min were bioassayed with the standard solutions prepared with M/15 phosphate buffer (pH 7.0) containing 66% ethanol. Each experiment was repeated three times in the same manner, and the values were averaged.

**(vi) Antibiotic concentrations in pouch exudates.** After subcutaneous injection of 20 ml of air into the backs of rats, 1 ml of 1% olive oil containing croton oil was injected into the pouch of each animal to obtain aseptic inflammation. A 20-mg amount of each antibiotic per kg was given intramuscularly on day 6 or 7 after formation of the pouch, and the exudates were collected at 0.5, 1, 2, 3, and 4 h. The antibiotic concentrations in the exudates were bioassayed with the standard solutions diluted with the exudates.

## RESULTS

**Antimicrobial activity.** In the experiments conducted as a part of this study, ceftezole showed a broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria, as did cefazolin and the other two cephalosporins tested (Table 1). Ceftezole was inactive against *Proteus vulgaris* IAM-1025 and *Pseudomonas aeruginosa* IAM-1095.

The susceptibilities of 354 strains isolated from patients to ceftezole are shown in Table 2. *Staphylococcus aureus, E. coli, Klebsiella pneumoniae,* and *P. mirabilis* were tested, using an inoculum of $10^8$ microbial cell units/ml. The MICs of ceftezole against 40 strains of *S. aureus* ranged from 0.2 to 3.13 μg/ml. The MIC of ceftezole for 21 (52.5%) of the 40 strains was 0.39 μg/ml. All strains tested were susceptible to ceftezole and to the other cephalosporins examined. The susceptibility of the strains was greatest for cephaloridine; cephalothin was the next most active. The susceptibility of these strains to ceftezole was almost the same as to cefazolin. The susceptibility of *S. aureus* to cephalosporins was greater than that of the gram-negative organisms tested. The susceptibility of 40 strains of *E. coli* to ceftezole was almost the same as that of cefazolin. The MICs ranged from 1.56 to >400 μg/ml and were most frequently 6.25 μg/ml (20 [50%] of the 40 strains). Four (10%) of the 40 test strains were highly resistant, the MICs being 100 μg/ml or greater. The antimicrobial activity of ceftezole against *E. coli* was almost the same as that of cefazolin and was markedly superior to that of cephalothin. The susceptibility of 60 strains of *K. pneumoniae* to ceftezole was almost the same as that of cefazolin. The MICs ranged from 1.56 to 400 μg/ml and were 3.13 μg/ml for 37 (61.7%) of the 60 strains. Five (8.3%) of the 60 strains were highly resistant, the MICs being 100 μg/ml or more. From these data, it can be assumed that the antimicrobial activity of cef-

TABLE 1. *Antimicrobial spectra of ceftezole and other cephalosporins*[a]

| Strain | MIC (μg/ml) of: | | | |
|---|---|---|---|---|
| | Ceftezole | Cefazolin | Cephaloridine | Cephalothin |
| *Staphylococcus aureus* 209P JC-1 | 0.2 | 0.39 | 0.1 | 0.39 |
| *S. aureus* Newman | 0.39 | 0.78 | 0.1 | 0.39 |
| *S. aureus* Terashima | 0.78 | 1.56 | 0.1 | 0.39 |
| *S. aureus* Smith | 0.2 | 0.39 | 0.025 | 0.2 |
| *S. aureus* ATCC-6538 P | 0.39 | 0.39 | 0.05 | 0.2 |
| *Bacillus subtilis* ATCC-6633 | 0.2 | 0.39 | 0.05 | 0.05 |
| *B. subtilis* PCI-219 | 0.2 | 0.39 | 0.05 | 0.05 |
| *Micrococcus luteus* PCI-1001 | 0.39 | 0.39 | 0.025 | 0.1 |
| *Streptococcus pneumoniae* III[b] | 0.2 | 0.39 | 0.1 | 0.2 |
| *S. pyogenes* S-23[b] | 0.2 | 0.2 | 0.05 | 0.1 |
| *S. pyogenes* A-S-8[b] | 0.2 | 0.2 | 0.05 | 0.1 |
| *S. faecalis* 6733[b] | 25 | 50 | 25 | 25 |
| *Corynebacterium diphtheriae* PW-8[b] | 0.05 | 0.1 | 0.05 | 1.56 |
| *C. diphtheriae* A-7[b] | 0.39 | 0.78 | 0.2 | 1.56 |
| *C. diphtheriae* M 406 MGL[b] | 0.39 | 0.78 | 0.2 | 0.39 |
| *Escherichia coli* NIHJ JC-2 | 6.25 | 3.13 | 6.25 | 25 |
| *E. coli* Yukitoshi | 6.25 | 3.13 | 6.25 | 12.5 |
| *E. coli* K-12 | 0.78 | 1.56 | 1.56 | 25 |
| *Klebsiella pneumoniae* NCTC-418 | 3.13 | 3.13 | 12.5 | 25 |
| *Proteus vulgaris* IAM-1025 | >100 | >100 | 100 | 100 |
| *Pseudomonas aeruginosa* IAM-1095 | >100 | >100 | >100 | >100 |
| *Salmonella typhi* T-287 | 0.78 | 1.56 | 3.13 | 1.56 |
| *S. typhi* O-901 | 1.56 | 1.56 | 3.13 | 3.13 |
| *S. paratyphi* A 1015 | 1.56 | 3.13 | 3.13 | 3.13 |
| *S. schottmuelleri* 8006 | 1.56 | 3.13 | 3.13 | 12.5 |
| *S. typhimurium* 1406 | 1.56 | 3.13 | 3.13 | 3.13 |
| *S. enteritidis* 1891 | 3.13 | 3.13 | 3.13 | 3.13 |
| *Shigella dysenteriae* A1 Shiga | 3.13 | 3.13 | 6.25 | 12.5 |
| *S. flexneri* 1a EW-8 | 3.13 | 6.25 | 6.25 | 6.25 |
| *S. flexneri* 1b Showa 15 | 6.25 | 3.13 | 3.13 | 12.5 |
| *S. flexneri* 2a Komagome B III | 3.13 | 3.13 | 3.13 | 12.5 |
| *S. flexneri* 3a EW-14 | 3.13 | 3.13 | 6.25 | 25 |
| *S. flexneri* 4a Saigon-Arai | 1.56 | 1.56 | 3.13 | 12.5 |
| *S. sonnei* I EW-33 | 1.56 | 0.78 | 1.56 | 6.25 |
| *S. sonnei* II EW-34 | 3.13 | 3.13 | 3.13 | 25 |

[a] Inoculum size, $10^8$ microbial cell units/ml.
[b] Supplemented with 10% rabbit blood.

TABLE 2. *Susceptibility of clinical isolates to ceftezole and other cephalosporins*

| Organism | Antibiotic | No. of organisms with an MIC (μg/ml) of: | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ≤0.05 | 0.1 | 0.2 | 0.39 | 0.78 | 1.56 | 3.13 | 6.25 | 12.5 | 25 | 50 | 100 | 200 | 400 | >400 |
| *Staphylococcus aureus*[a] (40 strains) | Ceftezole | | | 9 | 21 | 3 | 5 | 2 | | | | | | | | |
| | Cefazolin | | | 6 | 19 | 9 | 4 | 2 | | | | | | | | |
| | Cephaloridine | 10 | 14 | 3 | 6 | 2 | 3 | 2 | | | | | | | | |
| | Cephalothin | | | 14 | 19 | 7 | | | | | | | | | | |
| *Escherichia coli*[a] (40 strains) | Ceftezole | | | | | | 2 | 5 | 20 | 3 | 4 | 2 | | 1 | 2 | 1 |
| | Cefazolin | | | | | | 5 | 5 | 18 | 2 | 3 | 2 | | 3 | 1 | 1 |
| | Cephaloridine | | | | | | | 5 | 18 | 3 | 4 | 2 | 2 | 4 | | 2 |
| | Cephalothin | | | | | | | | 2 | 3 | 10 | 16 | 4 | | 1 | 4 |
| *Klebsiella pneumoniae*[a] (60 strains) | Ceftezole | | | | | | 5 | 37 | 4 | 2 | 5 | 2 | 3 | 1 | 1 | |
| | Cefazolin | | | | | | 4 | 31 | 9 | 7 | 4 | 1 | 3 | | 1 | |
| | Cephaloridine | | | | | | | 4 | 19 | 27 | 2 | 1 | 3 | 1 | 2 | 1 |
| | Cephalothin | | | | | | 1 | 1 | 12 | 30 | 8 | 2 | 4 | 2 | | |
| *Proteus mirabilis*[a] (41 strains) | Ceftezole | | | | | | | 1 | 11 | 23 | 6 | | | | | |
| | Cefazolin | | | | | | | 3 | 5 | 17 | 11 | 5 | | | | |
| | Cephalordine | | | | | | | | 1 | 9 | 25 | 6 | | | | |
| | Cephalothin | | | | | | | 2 | 8 | 18 | 11 | 2 | | | | |
| *P. vulgaris*[b] (21 strains) | Ceftezole | | | | | | | | | | | | 7 | 2 | 10 | 2 |
| | Cefazolin | | | | | | | | | | | | 2 | 4 | 6 | 9 |
| | Cephaloridine | | | | | | | | | | | | | 2 | 5 | 14 |
| | Cephalothin | | | | | | | | | | | | 2 | | 3 | 16 |
| *P. morganii*[b] (20 strains) | Ceftezole | | | | | | | | | | | 2 | 8 | 7 | 1 | 2 |
| | Cefazolin | | | | | | | | | | | | 3 | 10 | 3 | 4 |
| | Cephaloridine | | | | | | | | | | | | | 1 | 8 | 11 |
| | Cephalothin | | | | | | | | | | | | | | | 20 |
| *P. rettgeri*[b] (20 strains) | Ceftezole | | | | | 1 | 1 | | | 2 | 2 | 1 | 3 | 10 | | |
| | Cefazolin | | | | | 1 | 1 | 1 | | 1 | 2 | 1 | | 6 | 7 | |
| | Cephaloridine | | | | | | | | | 2 | 1 | | 1 | 16 | | |
| | Cephalothin | | | | | | | | | 1 | 1 | 1 | 1 | 1 | 2 | 13 |
| *P. inconstans B*[b] (20 strains) | Ceftezole | | | | | | 1 | | 3 | 4 | 6 | 3 | 3 | | | |
| | Cefazolin | | | | | | | 1 | 2 | 8 | 2 | 4 | 3 | | | |
| | Cephaloridine | | | | | | | | | | 3 | 2 | 8 | 4 | 3 | |
| | Cephalothin | | | | | | | | | 1 | 1 | 7 | 5 | 3 | 3 | |
| *Citrobacter*[b] (20 strains) | Ceftezole | | | | | 1 | | | | 1 | 6 | 4 | 4 | | | 4 |
| | Cefazolin | | | | | | 1 | | | | 5 | 2 | 4 | 3 | 1 | 4 |
| | Cephaloridine | | | | | | | 1 | | | | 2 | 4 | 7 | 3 | 3 |
| | Cephalothin | | | | | | | | 1 | 2 | | 8 | 2 | 2 | 1 | 4 |
| *Enterobacter aerogenes*[b] (19 strains) | Ceftezole | | | | | | 1 | 4 | 4 | 1 | | 3 | 3 | 1 | 2 | |
| | Cefazolin | | | | | | 2 | 4 | 3 | 1 | 1 | 1 | 3 | 2 | 2 | |
| | Cephaloridine | | | | | | | | 2 | 1 | 2 | 2 | 5 | 4 | 3 | |
| | Cephalothin | | | | | | | | | 3 | 4 | 3 | | 4 | | 5 |
| *E. cloacae*[b] (20 strains) | Ceftezole | | | | | | 1 | | 1 | | | | | | 6 | 12 |
| | Cefazolin | | | | | | 1 | | 1 | | | | | | 4 | 14 |
| | Cephaloridine | | | | | | | | 1 | | 1 | | | 1 | 4 | 13 |
| | Cephalothin | | | | | | | | | | 1 | 1 | | | | 18 |
| *Serratia marcescens*[b] (18 strains) | Ceftezole | | | | | | | | | | | | | | | 18 |
| | Cefazolin | | | | | | | | | | | | | | | 18 |
| | Cephaloridine | | | | | | | | | | | | | | 6 | 12 |
| | Cephalothin | | | | | | | | | | | | | | | 18 |
| *Acinetobacter*[b] (15 strains) | Ceftezole | | | | 1 | 1 | | | | 1 | | 1 | 1 | 2 | 1 | 7 |
| | Cefazolin | | | | 1 | | 1 | | | 1 | | | 2 | 2 | 2 | 6 |
| | Cephaloridine | | | | | 1 | | 1 | 1 | | 2 | 1 | 3 | 1 | 2 | 3 |
| | Cephalothin | | | | 1 | | | 1 | | 1 | | 1 | | 2 | 4 | 5 |

[a] Inoculum size, $10^8$ microbial cell units/ml.
[b] Inoculum size, $10^6$ microbial cell units/ml.

tezole against *K. pneumoniae* may be very similar to that of cefazolin and greater than that of other cephalosporins.

The MICs of ceftezole against 41 strains of *P. mirabilis* ranged from 3.13 to 25 μg/ml. The MICs of ceftezole were 12.5 μg/ml for 23 (57.5%) of the 41 strains. No strains resistant to more than 50 μg/ml (MIC) were found. Ceftezole was the most active of the cephalosporin derivatives.

Indole-positive *Proteus, Citrobacter, Enterobacter aerogenes, E. cloacae, Serratia marcescens,* and *Acinetobacter* were tested using inocula of $10^6$ microbial cell units/ml. The MICs of ceftezole against 21 strains of *P. vulgaris* ranged from 100 to >400 μg/ml, and those against 20 strains of *P. morganii* ranged from 50 to >400 μg/ml. Most of the strains were resistant to ceftezole and to the other cephalosporins tested. The MICs to ceftezole against 20 strains of *P. rettgeri* and 20 strains of *P. inconstans* B ranged from 0.78 to 200 μg/ml and from 1.56 to 100 μg/ml, respectively. The MICs of ceftezole against *Citrobacter* ranged from 0.78 to >400 μg/ml, and those against *E. aerogenes* (19 strains) ranged from 1.56 to 400 μg/ml. The susceptibility of these organisms was distributed widely. Two of the 20 strains of *E. cloacae* were susceptible to ceftezole (MIC, 1.56 and 6.25 μg/ml), but other strains were highly resistant (MIC, ≥400 μg/ml). The 18 strains of *S. marcescens* were all resistant to cephalosporins. Two or 3 out of 15 strains of *Acinetobacter* were susceptible to cephalosporins, but the remaining strains were resistant to cephalosporins.

To evaluate the effect of various test conditions on antimicrobial activity, the antimicrobial activity of ceftezole was compared by using five conventional agar media. As shown in Table 3, no abnormal fluctuations in antimicrobial activity of ceftezole and cefazolin against four test strains were noted in any of the five different media.

The effect of pH on the MIC of ceftezole was very similar to that of cefazolin (Table 3). *K. pneumoniae* 4106 was greatly influenced by the pH, i.e., an eightfold difference in MIC. *S. faecalis* 1215 was the least influenced.

Generally, the MICs of ceftezole against various test strains became correspondingly smaller when the inoculum size was reduced. Ceftezole closely resembled cefazolin in this respect.

When 10, 25, and 50% serum was added to each medium, there was little change in antimicrobial activity against most strains. The effect of serum on the antimicrobial activity of ceftezole was slightly less than that on the activity of cefazolin.

The bactericidal activity of ceftezole against *E. coli* NIHJ JC-2 in heart infusion broth was compared with that of cefazolin (Fig. 2).

Viable cell counts of *E. coli* NIHJ JC-2 decreased for 4 h after the start of incubation in broth containing one-fourth the MIC of ceftezole and, as in the case with cefazolin, increased 6 h after incubation. Under the same conditions, an increase of viable cell counts was more rapid for cephalothin than for ceftezole and cefazolin. At the MIC and four times the MIC, bactericidal activity of ceftezole was nearly the same as that of cefazolin and cephalothin, and viable cell counts did not increase even after prolonged incubation.

The hydrolysis of ceftezole and other cephalosporins by penicillinase type or cephalosporinase type β-lactamases isolated from various bacterial strains was investigated. The results were expressed as the relative hydrolysis rate of ceftezole and related cephalosporins in comparison with the hydrolysis rate of cefazolin under the same experimental conditions (Table 4). Under the conditions of these experiments, the hydrolysis rate of ceftezole was higher than that of cefazolin by penicillinase type β-lactamases isolated from *E. coli* 40 and two strains of *K. pneumoniae*. Among the five cephalosporinase type β-lactamases, the hydrolysis of ceftezole differed; two showed lower and three showed higher rates than did cefazolin. The results show that ceftezole is hydrolyzed slightly more easily than cefazolin.

Binding of ceftezole to human and animal serum protein was determined by the ultrafiltration method. Table 5 shows that there were differences in binding rates among different animal species. The binding of ceftezole to human serum protein was 86%, as was the case with cephalothin, higher than that of cephaloridine but lower than that of cefazolin. The binding of ceftezole to rat and rabbit serum protein was approximately 90%, i.e., almost the same as that of cefazolin, but the binding of ceftezole to mouse and dog serum protein was 27% and 19%, respectively, i.e., lower than that of cefazolin.

To determine the liberation of free drug from a protein-drug complex, antibiotic-serum solutions were diluted twofold with M/15 phosphate buffer (pH 7.0), and their antimicrobial potencies were compared.

As shown in Fig. 3, when ceftezole-serum solution was diluted fourfold with the phosphate buffer, the potency of ceftezole was completely recovered. When the cefazolin-serum so-

TABLE 3. *Influence of various factors on the antimicrobial activity of ceftezole and cefazolin*

| Factor | Antibiotic | MIC (μg/ml) of organism: | | | |
|---|---|---|---|---|---|
| | | A[a] | B | C | D |
| Medium | | | | | |
| HI[b] agar (Difco) | Ceftezole | 0.2 | 12.5 | 3.13 | 1.56 |
| Nutrient agar (Difco) | | 0.1 | 3.13 | 3.13 | 1.56 |
| BHI[b] agar (Difco) | | 0.1 | 3.13 | 6.25 | 3.13 |
| Trypticase soy agar (BBL) | | 0.1 | 3.13 | 6.25 | 3.13 |
| Mueller-Hinton agar (Difco) | | 0.1 | 3.13 | 6.25 | 3.13 |
| HI agar (Difco) | Cefazolin | 0.2 | 12.5 | 1.56 | 1.56 |
| Nutrient agar (Difco) | | 0.1 | 6.25 | 1.56 | 1.56 |
| BHI agar (Difco) | | 0.2 | 6.25 | 1.56 | 1.56 |
| Trypticase soy agar (BBL) | | 0.2 | 6.25 | 1.56 | 3.13 |
| Mueller-Hinton agar (Difco) | | 0.2 | 6.25 | 1.56 | 1.56 |
| pH | | | | | |
| 6.0 | Ceftezole | ≦0.05 | 6.25 | 1.56 | 1.56 |
| 7.0 | | 0.1 | 12.5 | 1.56 | 0.78 |
| 8.0 | | 0.2 | 12.5 | 1.56 | 1.56 |
| 9.0 | | 0.2 | 12.5 | 6.25 | 6.25 |
| 6.0 | Cefazolin | 0.1 | 6.25 | 1.56 | 3.13 |
| 7.0 | | 0.2 | 12.5 | 1.56 | 1.56 |
| 8.0 | | 0.2 | 12.5 | 1.56 | 1.56 |
| 9.0 | | 0.2 | 12.5 | 6.25 | 12.5 |
| Inoculum size | | | | | |
| $10^8$ | Ceftezole | 0.39 | 25 | 3.13 | 3.13 |
| $10^6$ | | 0.2 | 12.5 | 3.13 | 1.56 |
| $10^4$ | | 0.1 | 6.25 | 1.56 | 0.78 |
| $10^8$ | Cefazolin | 0.39 | 25 | 3.13 | 3.13 |
| $10^6$ | | 0.2 | 12.5 | 1.56 | 1.56 |
| $10^4$ | | 0.1 | 6.25 | 1.56 | 1.56 |
| Serum[c] | | | | | |
| 0 | Ceftezole | 0.2 | 12.5 | 1.56 | 0.78 |
| 10% | | 0.2 | 25 | 3.13 | 1.56 |
| 25 | | 0.39 | 25 | 1.56 | 3.13 |
| 50 | | 0.39 | 50 | 1.56 | 3.13 |
| 0 | Cefazolin | 0.2 | 12.5 | 3.13 | 0.78 |
| 10% | | 0.2 | 25 | 3.13 | 1.56 |
| 25 | | 0.39 | 50 | 1.56 | 3.13 |
| 50 | | 0.39 | 50 | 1.56 | 6.25 |

[a] Organism A, *S. aureus* 209-P JC-1; B, *S. faecalis* 1215; C, *E. coli* NIHJ JC-2; D, *K. pneumoniae* 4106.
[b] HI, Heart infusion; BHI, brain heart infusion.
[c] Serum, rabbit.

lution was diluted fourfold with the phosphate buffer, approximately 80% of its potency was recovered, but when diluted eightfold complete potency was recovered. The results obtained from cephalothin-serum solution diluted with the phosphate buffer in the same manner show that the liberation of cephalothin is less than that of ceftezole. The liberation of dicloxacillin was the least of the test antibiotics. From the above results, the binding of ceftezole in the protein-drug complex is thought to be comparatively loose.

The protective effect of ceftezole in experimental infection was studied in mice according to the procedures outlined in the experimental method. As shown in Table 6, the $ED_{50}$ of subcutaneously administered ceftezole against infections due to *E. coli* 362 was 0.92 mg/mouse, a protective effect which corresponded to that of cefazolin. The protective effect of ceftezole against infection produced by *E. coli* 362 was superior to that of cephalothin but inferior to that of cephaloridine. The $ED_{50}$ of subcutaneously administered ceftezole on infections produced by *K. pneumoniae* 410 was 0.05 mg/mouse, the effect being almost the same as that of cephaloridine. The protective effect against infection due to these organisms was superior to cephalothin and inferior to cefazolin. The $ED_{50}$ of ceftezole in infections produced by *P. mirabilis*

504 was 0.37 mg/mouse, the effect being nearly equal to that of cefazolin and cephaloridine and superior to that of cephalothin. The $ED_{50}$ of ceftezole in infections produced by *Citrobacter* 821 was 3.60 mg/mouse, which was nearly equal to that of cefazolin. The effect of ceftezole in infections produced by *Citrobacter* 821 was superior to that of cephaloridine and cephalothin.

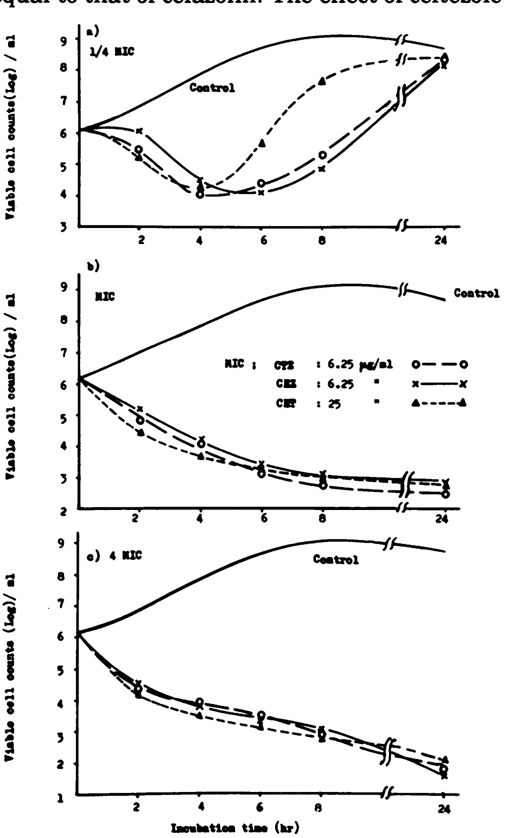


FIG. 2. *Bactericidal activity of ceftezole (CTZ), cefazolin (CEZ), and cephalothin (CET) against E. coli NIHJ JC-2.*

**Absorption and excretion. (i) Serum levels.** The concentrations of ceftezole in serum of SD strain rats after intramuscular injection of 20 mg/kg are shown in Fig. 4. The mean drug concentrations peaked at 33.8 μg/ml 15 min after injection and fell to 10.5 μg/ml within 1 h and to 0.9 μg/ml by 2 h. The peak concentrations and the concentrations at 30 min after ceftezole administration were about one-half those of cefazolin. The concentrations of ceftezole in serum were higher than those of the other cephalosporin derivatives tested, i.e., cephaloridine and cephalothin.

The mean concentrations of ceftezole in serum of beagle dogs after intramuscular injection of 20 mg/kg peaked at 49.7 μg/ml at 30 min and were 1.1 μg/ml at 6 h (Fig. 5). The concentrations of ceftezole in serum of dogs at the intervals specified in Fig. 5 (except 30 min) did not differ greatly from those of cefazolin and cephaloridine. The concentrations of cephalothin in serum however, were lower than those of ceftezole, cefazolin, and cephaloridine.

The concentrations of ceftezole in serum of monkeys after intramuscular injection of 20 mg/kg were compared with those of cefazolin, cephaloridine, and cephalothin. As shown in Fig. 6, the concentrations of ceftezole in serum were 40.0 μg/ml 30 min after injection, 1.3 μg/ml at 4 h, and not detectable at 6 h. After intramuscular injection of an equal dose of cephaloridine, the values were nearly the same. The concentrations of ceftezole in serum of monkeys were about one-half the levels of cefazolin in the early stage after administration but were higher than those of cephalothin, as was the case with rats.

TABLE 4. *Hydrolysis of ceftezole and other cephalosporins by different types of β-lactamases*[a]

| Substrate | Penicillinase type | | | Cephalosporinase type | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Escherichia coli* 40 | *Klebsiella pneumoniae*[b] 118 | *K. pneumoniae*[c] 164 | *E. coli* 36 | *Enterobacter aerogenes* 3 | *Citrobacter freundii* 15 | *Pseudomonas aeruginosa* 79 | *Proteus rettgeri* 8 |
| Cefazolin | 100[d] | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Ceftezole | 129 | 171 | 128 | 204 | 157 | 79 | 162 | 98 |
| Cephaloridine | 487 | 549 | 78 | 133 | 159 | 97 | 83 | 74 |
| Cephalothin | 200 | 147 | 82 | 710 | 479 | 95 | 404 | 120 |
| Cephalosporin C | 1,133 | 32 | 8 | 1,170 | 468 | 216 | 175 | 205 |

[a] β-lactamase was partially purified by gel filtration on Sephadex G-200. β-lactamase activity; initial hydrolysis velocity at the concentration of 50 μg/ml at 37 C by ultraviolet method.

[b] Indole-negative *K. pneumoniae*.

[c] Indole-positive *K. pneumoniae*.

[d] Relative activity (velocity of cefazolin = 100).

TABLE 5. *Extent of protein binding of ceftezole and other cephalosporins by centrifugal ultrafiltration technique*

| Antibiotic | Extent of protein bindings (%) in serum of: | | | | |
|---|---|---|---|---|---|
| | Humans | Dogs | Rabbits | Rats | Mice |
| Ceftezole | 86 | 19 | 97 | 90 | 27 |
| Cefazolin | 93 | 54 | 92 | 94 | 49 |
| Cephaloridine | 55 | 22 | 55 | 67 | 32 |
| Cephalothin | 86 | 44 | 86 | 87 | 57 |
| Dicloxacillin | 96 | 92 | 96 | 94 | 60 |

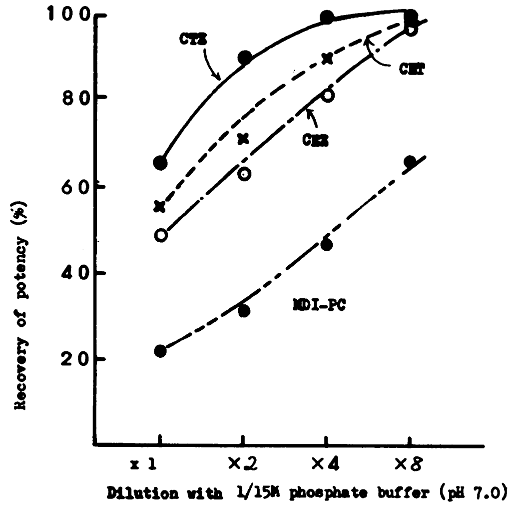


FIG. 3. *Release of free antibiotics from antibiotic-protein complex by buffer dilution. See legend to Fig. 2 for abbreviations.*

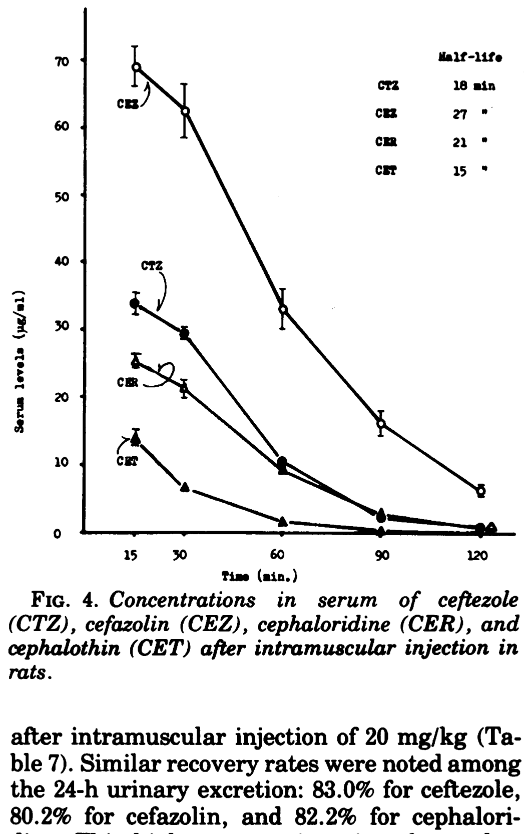


FIG. 4. *Concentrations in serum of ceftezole (CTZ), cefazolin (CEZ), cephaloridine (CER), and cephalothin (CET) after intramuscular injection in rats.*

As shown in Fig. 7, the concentrations of ceftezole in serum of seven healthy male volunteers after intramuscular injection of 500 mg peaked at a mean of 24.9 μg/ml at 15 min and were 17.5 μg/ml at 1 h, 10.0 μg/ml at 2 h, 2.6 μg/ml at 4 h, and not detectable in six subjects at 6 h. The half-life of ceftezole in serum of men was 56 min.

**(ii) Urinary excretion.** The urinary excretion of ceftezole in rats was determined at 24 h after intramuscular injection of 20 mg/kg (Table 7). Similar recovery rates were noted among the 24-h urinary excretion: 83.0% for ceftezole, 80.2% for cefazolin, and 82.2% for cephaloridine. This high recovery in urine shows that ceftezole is less readily metabolized than cepha-

TABLE 6. *Protective effect of ceftezole and other cephalosporins against experimental infections in mice*[a]

| Organism | | ED$_{50}$ (mg/mouse) of: | | | |
|---|---|---|---|---|---|
| | | Ceftezole | Cefazolin | Cephaloridine | Cephalothin |
| *Escherichia coli* | 362 | 0.92 (0.451-2.004)[b] | 0.63 (0.317-1.289) | 0.19 (0.087-0.447) | 7.03 (7.014-7.055) |
| *Klebsiella pneumoniae* | 410 | 0.05 (0.033-0.073) | 0.03 (0.010-0.039) | 0.06 (0.038-0.087) | 0.90 (0.591-1.339) |
| *Proteus mirabilis* | 504 | 0.37 (0.23-0.71) | 0.51 (0.35-0.78) | 0.45 (0.23-0.96) | 1.53 (0.79-3.08) |
| *Citrobacter* | 821 | 3.60 (2.365-5.357) | 4.19 (2.787-6.409) | 5.00 (3.31-7.68) | >20 |

[a] All animals were challenged by the intraperitoneal route; drugs were administered subcutaneously.
[b] Numbers in parentheses = 95% confidence limit.

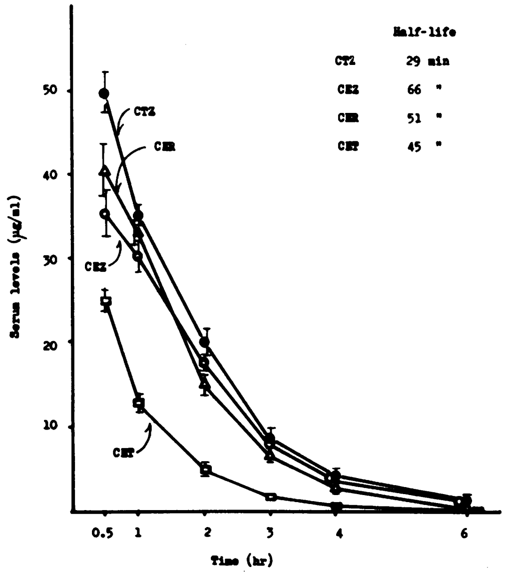


FIG. 5. *Concentrations in serum of ceftezole (CTZ), cefazolin (CEZ), cephaloridine (CER), and cephalothin (CET) after intramuscular injection in dogs.*

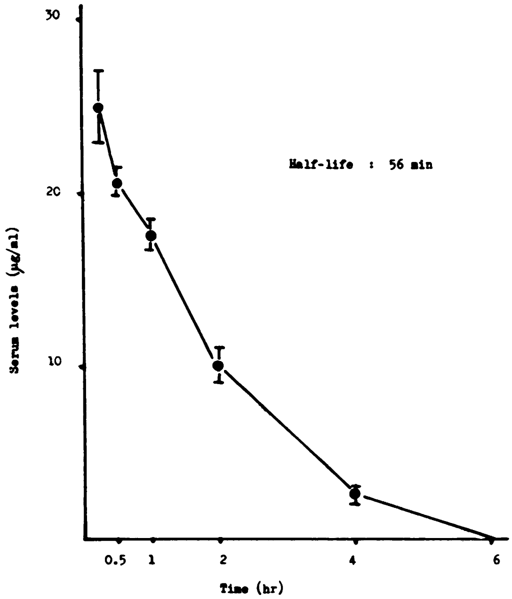


FIG. 7. *Concentrations in serum of ceftezole after intramuscular injection in seven healthy human volunteers.*

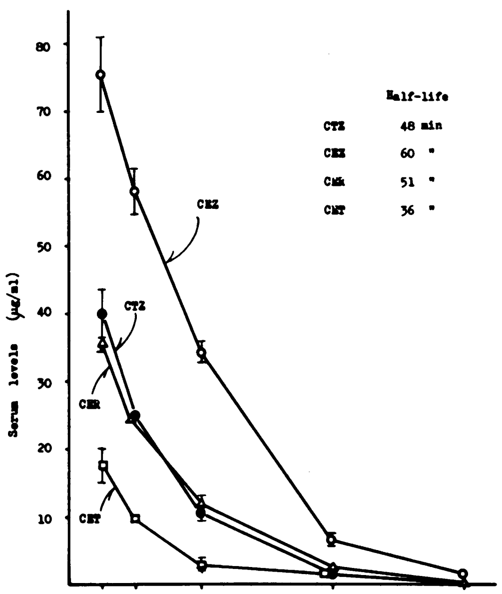


FIG. 6. *Concentrations in serum of ceftezole (CTZ), cefazolin (CEZ), cephaloridine (CER), and cephalothin (CET) after intramuscular injection in monkeys.*

lothin (22.1%).

The urinary excretion in beagle dogs for 24 h after intramuscular injection of 20 mg/kg averaged 73.5% of the administered dose for ceftezole, 80.1% for cefazolin, and 78.9% for cephaloridine (Table 8). These cephalosporin derivatives show high recovery in urine. No statistically significant differences were noted among these antibiotics. The urinary excretion of cephalothin, however, was 35.1%, significantly lower than that of ceftezole.

The urinary excretion of ceftezole in monkeys for 24 h after intramuscular injection of 20 mg/kg was compared with those of cefazolin, cephaloridine, and cephalothin (Table 9). The urinary excretion was 72.7% for ceftezole, 80.3% for cefazolin, and 74.4% for cephaloridine. No significant differences in urinary excretion were observed among these antibiotics, as was the case with other test animals. The urinary excretion of cephalothin was lower (49.4%).

The 24-h urinary excretion of ceftezole in seven healthy male volunteers after a single intramuscular injection of 500 mg averaged 86.6% (Table 10). The results show that ceftezole is stable in the human body. About 87.5% of the total excretion was recovered within 3 h after intramuscular injection. The urinary excretion of ceftezole was rapid. The urinary levels of ceftezole peaked at a mean of 2,390 μg/ml at 1 h and averaged 244 μg/ml (80 to 440 μg/ml) at 3 to 6 h. These levels were high enough to inhibit the growth of susceptible organisms.

An investigation was done by the thin-layer

TABLE 7. *Urinary excretion of ceftezole and other cephalosporins after intramuscular injection in rats*

| Antibiotic | Urinary excretion at: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0-3 h | | 3-6 h | | 6-24 h | | Total | |
| | μg/ml | % | μg/ml | % | μg/ml | % | mg | % |
| Ceftezole | 1,900 | 72.0 | 168 | 5.80 | 4.50 | 0.53 | 3.41 | 83.0 |
| ($n$ = 10) | (368)[a] | (2.7) | (32.0) | (1.20) | (1.50) | (0.15) | (0.10) | (1.9) |
| Cefazolin | 1,580 | 75.7 | 171 | 4.05 | 4.04 | 0.44 | 3.59 | 80.2 |
| ($n$ = 10) | (195) | (1.9) | (24.7) | (0.50) | (0.90) | (0.09) | (0.06) | (1.7) |
| Cephaloridine | 1,430 | 79.7 | 83.6 | 2.2 | 2.02 | 0.31 | 3.54 | 82.2 |
| ($n$ = 10) | (125) | (2.1) | (12.6) | (0.30) | (0.35) | (0.05) | (0.12) | (2.2) |
| Cephalothin | 391 | 21.8 | 12.2 | 0.3 | 0.25 | 0.02 | 0.95 | 22.1 |
| ($n$ = 10) | (60.4) | (1.5) | (3.2) | (0.07) | (0.15) | (0.01) | (0.06) | (1.5) |

[a] Numbers in parentheses = standard error.

TABLE 8. *Urinary excretion of ceftezole and other cephalosporins after intramuscular injection in dogs*

| Antibiotic | Urinary excretion at: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0-3 h | | 3-6 h | | 6-24 h | | Total | |
| | μg/ml | % | μg/ml | % | μg/ml | % | mg | % |
| Ceftezole | 2,480 | 54.1 | 896 | 15.9 | 119 | 3.6 | 170 | 73.5 |
| ($n$ = 6) | (447)[a] | (3.5) | (215) | (2.8) | (48.1) | (1.3) | (6.7) | (2.1) |
| Cefazolin | 3,030 | 64.5 | 718 | 14.2 | 29.1 | 1.4 | 175 | 80.1 |
| ($n$ = 5) | (743) | (2.8) | (95) | (0.6) | (4.8) | (0.1) | (8.4) | (2.9) |
| Cephaloridine | 2,040 | 70.1 | 388 | 7.8 | 19.6 | 1.0 | 177 | 78.9 |
| ($n$ = 5) | (344) | (4.7) | (30) | (0.6) | (3.2) | (0.1) | (8.6) | (4.8) |
| Cephalothin | 622 | 33.1 | 71 | 1.5 | 6.7 | 0.4 | 76.3 | 35.1 |
| ($n$ = 5) | (54) | (2.3) | (25) | (0.3) | (3.9) | (0.2) | (6.0) | (2.0) |

[a] Numbers in parentheses = standard error.

TABLE 9. *Urinary excretion of ceftezole and other cephalosporins after intramuscular injection in monkeys*

| Antibiotic | Urinary excretion at: | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0-2 h | | 2-4 h | | 4-6 h | | 6-24 h | | Total | |
| | μg/ml | % | μg/ml | % | μg/ml | % | μg/ml | % | mg | % |
| Ceftezole | 2,020 | 52.7 | 1,020 | 15.8 | 354 | 4.0 | 3.0 | 0.2 | 108 | 72.7 |
| ($n$ = 6) | (859)[a] | (2.8) | (132) | (0.8) | (121) | (1.4) | (0.9) | (0.04) | (4.8) | (1.4) |
| Cefazolin | 1,410 | 45.0 | 1,240 | 28.6 | 139 | 4.6 | 48.6 | 2.2 | 120 | 80.3 |
| ($n$ = 3) | (139) | (8.3) | (441) | (6.8) | (38) | (0.7) | (17.5) | (0.7) | (10) | (0.5) |
| Cephalori- | 764 | 58.6 | 780 | 15.8 | 218 | 2.2 | 6.3 | 0.3 | 115 | 74.4 |
| dine | (37) | (2.9) | (234) | (3.2) | (75) | (0.6) | (2.0) | (0.1) | (10) | (1.5) |
| ($n$ = 3) | | | | | | | | | | |
| Cephalothin | 602 | 38.8 | 405 | 9.8 | 25.4 | 0.83 | 0.25 | 0.02 | 77.2 | 49.4 |
| ($n$ = 3) | (92) | (4.7) | (63.3) | (4.1) | (9.1) | (0.27) | (0.03) | (0.01) | (8.2) | (2.7) |

[a] Numbers in parentheses = standard error.

chromatographic and bioautographic methods to confirm whether active substances in the urine of animals and human volunteers after intramuscular injection of ceftezole included unchanged ceftezole. Figure 8 shows the bioautograms. Only one active substance was found in urine samples, the $R_f$ value of which corresponded to intact ceftezole.

**(iii) Biliary excretion.** The biliary excretion of ceftezole in rats for 24 h after intramuscular injection of 20 mg/kg was compared with that of the other cephalosporin derivatives (Table 11). The biliary excretion was 4.4% of the administered dose for ceftezole and 9.9% for cefazolin. The excretion of ceftezole was lower than that of cefazolin but was higher than that of cephaloridine and cephalothin.

**(iv) Tissue distribution.** The tissue distribution of ceftezole after intramuscular injection of 20 mg/kg was determined (Table 12). Ceftezole

TABLE 10. *Urinary excretion of ceftezole after intramuscular injection in healthy human volunteers*

| Volunteer | | Urinary excretion at: 0-1 h | | 1-2 h | | 2-3 h | | 3-6 h | | 6-24 h | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | Wt (kg) | μg/ml | % | μg/ml | % | μg/ml | % | μg/ml | % | μg/ml | % | mg | % |
| A | 75 | 887 | 24.0 | 887 | 26.2 | 730 | 17.7 | 395 | 20.0 | 11.7 | 3.86 | 459 | 91.8 |
| B | 69 | 1,150 | 39.8 | 897 | 31.6 | 540 | 13.8 | 144 | 7.34 | 3.40 | 0.86 | 467 | 93.4 |
| C | 60 | 1,440 | 41.4 | 835 | 25.0 | 690 | 12.3 | 185 | 5.00 | 2.74 | 1.06 | 424 | 84.8 |
| D | 60 | 8,000 | 38.4 | 4,630 | 33.4 | 1,380 | 8.0 | 440 | 7.66 | 4.30 | 0.82 | 441 | 88.3 |
| E | 56 | 1,370 | 29.0 | 1,170 | 30.0 | 780 | 12.3 | 358 | 17.2 | 3.00 | 0.83 | 447 | 89.3 |
| F | 55 | 1,710 | 30.4 | 960 | 27.2 | 290 | 12.4 | 80.0 | 7.64 | 2.50 | 0.91 | 393 | 78.6 |
| G | 69 | 2,170 | 41.6 | 670 | 24.6 | 305 | 11.7 | 105 | 1.55 | 1.48 | 0.29 | 399 | 79.7 |
| Mean | | 2,390 | 34.9 | 1,440 | 28.3 | 674 | 12.6 | 244 | 9.48 | 4.16 | 1.23 | 433 | 86.6 |
| | | (948)[a] | (2.7) | (535) | (1.3) | (139) | (1.1) | (56.5) | (2.50) | (1.29) | (0.44) | (10.8) | (2.17) |

[a] Numbers in parentheses = standard error.

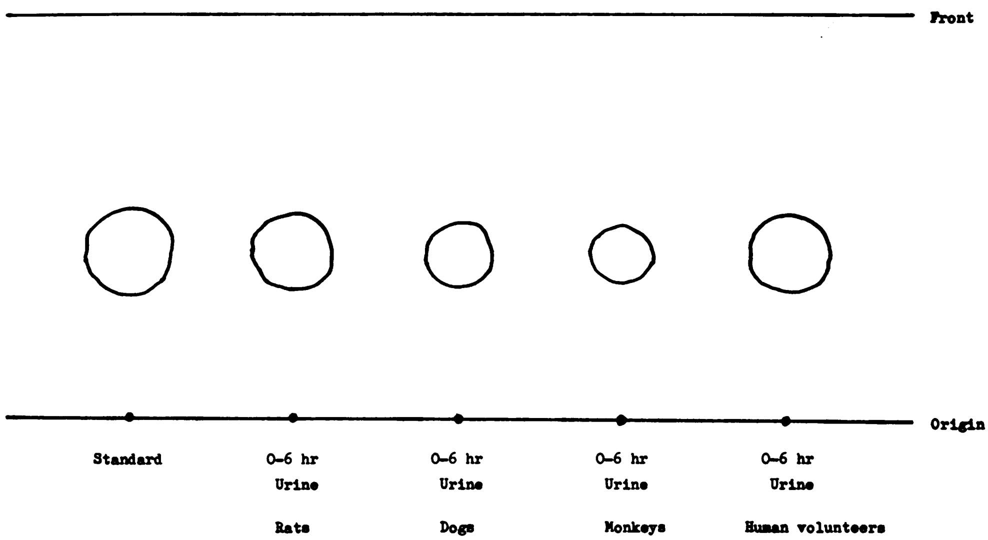


FIG. 8. *Bioautograms of urine of rats, dogs, monkeys (20 mg/kg), and healthy human volunteers (500 mg) after intramuscular injection of ceftezole.*

TABLE 11. *Biliary excretion of ceftezole and other cephalosporins after intramuscular injection in rats*

| Antibiotic | Biliary excretion at: 0-3 h | | 3-6 h | | 6-24 h | | Total | |
|---|---|---|---|---|---|---|---|---|
| | μg/ml | % | μg/ml | % | μg/ml | % | mg | % |
| Ceftezole | 67.4 | 4.3 | 1.6 | 0.08 | ND[a] | ND | 0.194 | 4.4 |
| ($n$ = 10) | (10.2)[b] | (0.6) | (0.4) | (0.02) | | | (0.029) | (0.6) |
| Cefazolin | 141 | 9.4 | 12.3 | 0.5 | 0.05 | 0.01 | 0.430 | 9.9 |
| ($n$ = 10) | (17.1) | (1.3) | (1.3) | (0.05) | (0.02) | (0.00) | (0.06) | (1.3) |
| Cephaloridine | 6.0 | 0.37 | 1.23 | 0.05 | 0.05 | 0.01 | 0.017 | 0.44 |
| ($n$ = 10) | (0.72) | (0.05) | (0.14) | (0.01) | (0.02) | (0.00) | (0.002) | (0.06) |
| Cephalothin | 12.0 | 0.90 | 0.05 | 0.0 | 0.05 | 0.01 | 0.041 | 0.91 |
| ($n$ = 10) | (1.8) | (0.14) | (0.02) | (0.00) | (0.02) | (0.00) | (0.007) | (0.14) |

[a] ND, Not detected.
[b] Numbers in parentheses = standard error.

was detected in all the tissue samples. The peak tissue levels at 15 to 30 min were 71.3 μg/g in the kidney, 9.8 μg/g in the liver, 8.7 μg/g in the lung, 5.1 μg/g in the heart, and 2.3 μg/g in the spleen. When these tissue levels were compared with those of cefazolin, all were lower

TABLE 12. *Tissue distribution of ceftezole and other cephalosporins after intramuscular injection in rats*

| Antibiotic | Time (min) | Tissue levels (μg/g) in: Liver | Kidneys | Lungs | Heart | Spleen | Concn in serum (μg/ml) |
|---|---|---|---|---|---|---|---|
| Ceftezole | 15 | 9.2 | 71.3 | 8.7 | 5.1 | 2.3 | 33.1 |
| (n = 6) | 30 | 9.8 | 48.0 | 6.4 | 4.8 | 1.9 | 29.0 |
| | 60 | 4.8 | 22.8 | 3.5 | 2.2 | 0.6 | 13.5 |
| | 90 | 1.4 | 6.9 | 0.9 | 0.5 | ND[a] | 3.8 |
| Cefazolin | 15 | 11.0 | 53.4 | 13.2 | 8.3 | 3.2 | 63.8 |
| (n = 9) | 30 | 16.3 | 45.4 | 10.9 | 5.8 | 2.7 | 50.0 |
| | 60 | 9.5 | 33.4 | 6.6 | 4.7 | 1.8 | 30.8 |
| | 90 | 5.0 | 13.6 | 3.5 | 2.1 | 1.0 | 12.5 |
| Cephaloridine | 30 | 8.6 | 70.3 | 6.8 | 3.0 | 2.1 | 19.6 |
| (n = 9) | 60 | 6.8 | 27.9 | 3.3 | 1.4 | 1.1 | 7.8 |
| | 90 | 4.9 | 8.7 | 1.3 | 0.7 | 1.0 | 2.7 |
| Cephalothin | 30 | 2.4 | 8.5 | 0.6 | 0.3 | ND | 5.5 |
| (n = 9) | 60 | 0.4 | 1.6 | 0.1 | ND | ND | 1.4 |
| | 90 | ND | ND | ND | ND | ND | 0.3 |

[a] ND, Not detected.

except renal levels at 15 to 30 min after injection. All the tissue levels of ceftezole were higher than those of cephalothin.

**(v) Levels in rat pouch exudate.** Ceftezole levels in the exudate of pouches with aseptic inflammation after intramuscular injection of 20 mg/kg to SD strain rats were compared with cefazolin levels (Fig. 9a and b). Ceftezole and cefazolin levels on day 6 and 7 after formation of pouches differed, and these levels decreased with the lapse of time. The levels of ceftezole in the exudate were slightly lower than those of cefazolin under all conditions.

## DISCUSSION

No significant differences in antimicrobial activity of ceftezole and cefazolin against the clinical isolates tested were noted. The antimicrobial activity of ceftezole against most often encountered gram-negative bacilli, *E. coli*, *P. mirabilis*, and *K. pneumoniae* was higher than any of the other known cephalosporins. Among gram-negative bacilli considered to be resistant to cephalosporin derivatives, such as *P. rettgeri*, *P. inconstans* B, *Citrobacter*, and *E. aerogenes*, susceptibility to ceftezole and cefazolin was considerably higher with small sizes of inocula.

Apparent differences in serum-protein binding were observed between ceftezole and cefazolin. These differences were not great, but the protein binding of ceftezole was estimated to be looser than that of cefazolin on the basis of the dissociation rate of the active substance from

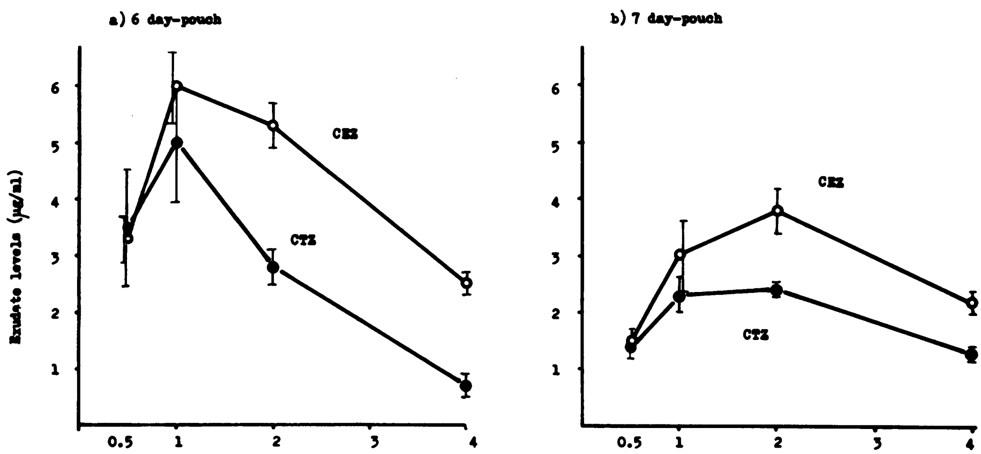


FIG. 9. *Exudate levels of ceftezole (CTZ) and cefazolin (CEZ) after intramuscular injection in rats with granuloma pouch.*

the binding conjugation by the buffer dilution (5). The influence of protein binding of antibiotics on clinical effect has been discussed but still remains controversial. The clinical trials of both drugs, which are similar in in vitro antimicrobial activity but different in protein binding, may be useful to elucidate the influence of serum-protein binding on clinical effect.

In rats, renal levels of ceftezole were markedly higher than those of cefazolin in the early stage after administration.

The ratio of renal levels to concentrations in serum of ceftezole was 2.15 at 15 min, 1.65 at 30 min, and 1.69 at 60 min. The renal levels of ceftezole at each time were 1.6 to 2 times the concentrations in serum. High levels of ceftezole in the kidneys were maintained, whereas the renal levels of cefazolin at each interval were nearly the same as concentrations in serum: 0.84 times at 15 min, 0.91 times at 30 min, and 1.08 times at 60 min. The ratios of all the tissue levels to concentrations in serum, except kidneys, were higher than those of cefazolin. These results indicate that the diffusion rates of ceftezole into the tissues from the blood is higher than that of cefazolin and may be explained by the weaker serum-protein binding of ceftezole. Furthermore, the extremely high renal levels of ceftezole in the early stage after administration may suggest that renal excretion of ceftezole is faster than that of cefazolin. The half-life of concentrations of ceftezole in serum of healthy volunteers after intramuscular injection was about 1 h, one-half that of cefazolin.

This new antibiotic, which equals cefazolin in antimicrobial activity and exceeds cefazolin in speed of renal clearance, may be useful in treating patients with renal dysfunction, when accumulation of an antibiotic is an important consideration.

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